TUBERCULOSIS

NIAID plays a leading role in the NIH tuberculosis (TB) research program. Worldwide TB case rates are increasing primarily because of the ongoing HIV/AIDS epidemic and the development of multidrug-resistant (MDR) strains of *Mycobacterium tuberculosis (M.tb),* the pathogen that causes TB. In response to ongoing public health concerns about the global TB epidemic, NIAID has increased its TB research portfolio steadily over the past decade. The World Health Organization (WHO) estimates that there are approximately 8 million new TB cases annually, with 2 million deaths. This toll makes TB the leading cause of death from a single infectious pathogen worldwide, killing more people than AIDS and malaria combined. Approximately one-third of the world's population is infected with *M.tb*, and 1 in 10 of these individuals likely will develop active TB disease in his or her lifetime. However, persons with a weakened immune system, such as those infected with HIV, have a much higher chance of developing active TB and of dying from this disease. If current trends continue, an estimated 1 billion people will be newly infected by the year 2020, approximately 200 million people will develop active TB, and 35 million will die.[69]

NIAID supports a broad TB research program through its extramural Division of Microbiology and Infectious Diseases (DMID), the Division of AIDS (DAIDS), the Division of Allergy, Immunology and Transplantation (DAIT), and through its intramural program, with particular emphasis on the following areas:

- Basic biology and pathogenesis of *M.tb*, host-pathogen interaction, and host response to TB in animal models and humans. The goal is to understand how the immune system recognizes and responds to bacteria such as *M.tb*, hidden within host cells, and to support research on antigen presentation and stress molecule induction as they relate to activation of cell-mediated immunity to intracellular pathogens.
- Research into the various stages of TB, including persistent, asymptomatic infection with *M.tb* (latency), reactivation, and progression to active TB. This research strives to identify immune system genes that are activated by mycobacterial infection, especially genes that encode soluble proteins that might be relevant to the development of TB vaccines or therapies, and to elucidate T-lymphocyte recognition of mycobacterial lipid antigens, the role of various cell populations in combating *M.tb* infection, and the function of biological oxidants in protective immune processes.
- Development and testing of vaccines, chemotherapeutics, and diagnostics. This focus includes efforts to promote vaccine-relevant research to identify dominant mycobacterial antigens and novel adjuvants that induce protective cellular immune responses, as well as research on the development of immunologic reagents for early diagnosis and monitoring of disease.
- Development of improved tools for epidemiologic studies.
- Mycobacterial genomics and postgenomic analyses.

Consistent funding support over the past 12 years and the inclusion of applied research into the characterization and prevention of MDR-TB in NIAID's Biodefense and Re-emerging Infectious Diseases research agenda have allowed the Institute to support a number of initiatives and partnerships that have markedly expanded the community of TB researchers. Furthermore, funding initiatives to encourage participation of small companies in the development of new healthcare interventions for TB have resulted in a significant number of new product development projects and approaches, many of which are

expected to be validated within the next several years.

NIAID's extramural TB research program currently supports more than 200 grants and 12 contracts for basic, applied, and clinical TB research. The contracts are designed to fill critical gaps in applying fundamental research findings to the development of new healthcare interventions for TB and to provide tools for the conduct of high quality, ethical human clinical studies and trials. NIAID offers TB researchers contracts to study specific mycobacteria, as well as access to more general scientific contract resources, such as mycobacterial microarrays provided by the Pathogen Functional Genomics Resource Center *(www.niaid.nih.gov/dmid/genomes/pfgrc/guidelines.htm*). For access to genome data, see *www.tigr.org/tdb/mdb/mdbinprogress.html*. Initiatives to encourage public-private partnerships have resulted in several grant awards focusing on the preclinical development of novel diagnostics, drugs, and vaccine candidates that are expected to enter clinical trials within the next few years.

The development of improved TB vaccines, which are crucial to the long-term control of TB worldwide, is a high priority. In December 2003, NIAID and the U.S. Food and Drug Administration (FDA) Center for Biologics Evaluation and Research sponsored a workshop to outline U.S. regulatory requirements for the development and human testing of new TB vaccines (*www.niaid.nih.gov/dmid/meetings/tbvacc.htm*). Clinical trials of two new TB vaccines that were developed with NIAID support began in 2004. One is a recombinant version of the bacillus Calmette-Guerin vaccine, developed by investigators at the University of California, Los Angeles; the other is an adjuvant-peptide fusion vaccine developed by Corixa Corporation.

Through the DMID-supported Tuberculosis Research Materials and Vaccine Testing contract with Colorado State University (*www.cvmbs.colostate.edu/microbiology/tb/top.htm*), NIAID provides TB research reagents and preclinical vaccine testing services to qualified investigators throughout the world. By the end of FY 2004, more than 150 new TB vaccine candidates had been tested under this contract. One of these has recently entered human clinical trials and several others are progressing through various stages of preclinical development. Through the Millennium Vaccine contract (Infectious Disease Research Institute, Seattle, Washington), NIAID provides funding to the private sector for the development of improved TB vaccines using existing technology platforms.

In 1994, NIAID established the Tuberculosis Research Unit (TBRU) at Case Western Reserve University (*www.cwru.edu/affil/tbru/index.htm*). The grant was recompeted in 1999. The DMID-funded TBRU provides knowledge, tools, and technologies to improve human clinical trials in TB and the capability to conduct clinical studies for the evaluation of new or improved vaccines, therapeutics, and diagnostics. Activities of the TBRU are coordinated with other major organizations involved in TB research, including the Centers for Disease Control and Prevention, U.S. Agency for International Development, FDA, WHO, Global Alliance for TB Drug Development, International Union Against Tuberculosis and Lung Disease, and interested industrial partners.

Development of new drugs and improved and shortened therapeutic regimens to treat and prevent active TB is a longstanding activity within NIAID. The Southern Research Institute (SRI) maintains, under contract with NIAID, the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) to acquire drug candidates for screening against virulent *M.tb.* The SRI also maintains a computerized chemical database of candidate structures, coordinates and distributes compounds for evaluation *in vitro* and in animal models, and reports data

to compound suppliers. TAACF has contacted more than 3,500 chemists throughout the world seeking candidate anti-TB compounds. TAACF has received more than 79,000 compounds from academic and private-sector investigators, principally in the United States and Europe, with growing involvement of scientists in Africa, Asia, Australia, South America, and other regions.[70]

NIAID supports a contract for the Tuberculosis Drug Screening program to provide high-throughput screening services to discover new antimicrobials. The facility supported under this contract provides the capability to test large chemical libraries of compounds for activity against specific biochemical drug targets and against growing microorganisms. In addition to supporting *in vitro* evaluation of compounds from chemical repositories, in FY 2005, DAIDS awarded a research grant to stimulate preclinical research of a new class of therapeutics against TB, in the context of HIV/AIDS.

DAIDS also supports the Animal Model Testing of TB Drugs contract, which provides critical support for investigator-initiated drug discovery, stimulates private sector sponsorship of new drugs, performs comparison and confirmatory studies, and provides information for selection of drug candidates for design of clinical studies. In 2004, DAIDS funded the Pharmacokinetics and Pharmacodynamics Animal Model contract. This contract supports a central facility to identify and evaluate novel compounds for their basic pharmacology and efficacy characteristics, provides critical support for investigator-initiated drug discovery, stimulates private-sector sponsorship of new drugs, performs comparison and confirmatory studies from different sponsors, and provides information for the selection of antimicrobial drug candidates for designing clinical studies. DAIDS continues to support the Tuberculosis Technology Transfer contract, which spurs the translation of anti-tuberculosis discoveries into candidates for development and commercialization (*www.newtbrx.org*).

DAIDS supports clinical trials of new treatment and prevention strategies for tuberculosis in the setting of HIV/AIDS. These investigations are being conducted in countries with a high burden of both TB and HIV. The interactions of these two infections are associated with high mortality, particularly in African nations. DAIDS continues to support research projects designed to develop effective and sustainable clinical management strategies to improve care and foster integration of research on HIV and co-infection pathogens, including tuberculosis. In addition, the NIAID Comprehensive International Program of Research on AIDS supports research studies that address important public health research questions in high-burden countries.

NIAID participates in a public-private partnership, the Global Alliance for Tuberculosis Drug Development (*www.tballiance.org*). NIAID, together with WHO, the Rockefeller Foundation, and other international organizations in the TB Alliance, is dedicated to encouraging new therapeutic advances in the absence of industrial sponsorship. Recently, in partnership with the TB Alliance and through the TAACF contract, NIAID contributed to the preclinical safety and efficacy testing in animal models of a novel antibiotic, PA-824. In addition, increased funding through Small Business Innovation Research grants has promoted development and evaluation of new tools for treating and preventing tuberculosis.

DAIT supports a number of individual research projects concerned with basic mechanisms of immunity to *M.tb*. DAIT's research goals and objectives on *M.tb* are to

- Understand how the immune system recognizes and responds to bacteria such as *M.tb*, hidden within host cells, and support research on antigen presentation and stress molecule induction as they relate to activation of cell-mediated immunity to intracellular pathogens;

- Promote vaccine-relevant research to identify dominant mycobacterial antigens and novel adjuvants that induce protective cellular immune responses;
- Promote research on the development of immunologic reagents for early diagnosis and monitoring of disease; and
- Support research on the identification of immune system genes that activate in response to mycobacterial infection, especially genes that encode soluble proteins that might be relevant to the development of TB vaccines or therapies.

Research topics include T-lymphocyte recognition of mycobacterial lipid antigens, the role of various cell populations in combating *M.tb* infection, and the function of biological oxidants in protective immune processes.

DAIT sponsors several projects that support research on TB as well as other infectious diseases such as hepatitis C, malaria, and HIV. Under the Application of Data on Human Leukocyte Antigen (HLA) to the Improvement of Vaccines program, DAIT supports the HLA Ligand/Motif Online Database, a Web-based, searchable database of human major histocompatibility complex (MHC) molecules and peptide ligands. The database specifies amino acid sequences of peptides derived from viral, bacterial, parasite, and human proteins in association with human class I or class II MHC molecules. This resource enables users to search for specific human MHC/peptide combinations or to determine ligand amino acid motifs that will facilitate their research. Support is provided under a NIAID contract to the University of Oklahoma (*hlaligand.ouhsc.edu/index_2.html*)

The NIH Tetramer Facility provides custom synthesis and distribution of soluble MHC-peptide tetramer reagents that can be used to detect antigen-specific T cells. These reagents are provided to approved investigators, who supply the purified peptides and cover the cost of shipping these peptides and the synthesized tetramer reagents. The Tetramer Facility contract covers the cost of tetramer production and validation/quality control. The Facility serves as both a tetramer production facility and as a research and development facility for the generation of novel tetramer reagents. The contract supports the continuation of this reagent resource, which provides custom-made MHC class I, MHC class I-like, and MHC class II tetramers to the research community. More information about this facility can be found at *www.niaid.nih.gov/reposit/tetramer/index.html*. The National Cancer Institute also provides funding for the Tetramer Facility.

Tuberculosis research within NIAID's Division of Intramural Research (DIR) focuses on understanding the molecular pathology of TB with an eye to developing new interventions that will make a substantial difference in disease control. The DIR TB research portfolio forms a spectrum from laboratory to clinic-based studies. DIR scientists collaborate with a wide variety of academic and industrial partners as well as other government agencies to maximize the likelihood that laboratory discoveries will make a difference to clinical practice.

DIR scientists are engaged directly in the identification, design, and synthesis of promising new drug candidates from several chemical series. Nitroimidazoles such as PA-824, which is currently in clinical trials, are a major focus because they have the potential to be effective against both the drug-resistant and drug-sensitive forms of TB. In 2000, DIR researchers working in collaboration with scientists at Pathogenesis Corporation were part of the team that first reported the activity of this series of molecules. DIR scientists have partnered with the Novartis Institute for Tropical Diseases in Singapore, a research institute working on neglected diseases (*www.nitd.novartis.com*), to design, synthesize, and test nitroimidazoles other than PA-824

while working to understand the key molecular mechanism of nitroimidazoles. In another structural class, DIR scientists synthesized SQ-109, a more potent, second-generation analog of ethambutol, in partnership with Sequella, Inc (*www.sequella.com*). This molecule is also advancing towards clinical trials.

DIR TB researchers maintain an active program of genome-scale analyses as applied to drug development. These types of approaches allow both the rapid identification of the target of new antimycobacterial agents and identification of promising new targets for drug design. These techniques are being applied to shorten the course of chemotherapy in an approach known as "chemical genomics." Partnering with DAIDS and scientists at the National Institute for Pharmaceutical Research and Development in Abuja, Nigeria, DIR is working to transfer knowledge of the application of genomic information and structure-based drug discovery to scientists in TB-endemic sub-Saharan Africa. DIR scientists are also partners with the Korean Ministry of Science and Technology and the Korea Research Institute for Chemical Technology in Taejon, South Korea, in a newly established International Center for TB Drug Development.

In an international partnership that includes scientists from the United Kingdom, United States, Singapore, South Korea, and Mexico, DIR researchers have a leading role in a $20 million grant awarded jointly by the Bill and Melinda Gates Foundation and the Wellcome Trust as part of the Grand Challenges in Global Health initiative (*www.grandchallengesgh.org*) to develop new therapeutics for the treatment of latent tuberculosis infections. In this effort, DIR works closely with collaborators at the International Tuberculosis Research Center, which is funded by DIR and the Korean Ministry of Health and located at the National Masan Tuberculosis Hospital in South Korea. This grant will make it possible for DIR researchers to perform key experiments to identify vulnerable targets in TB bacilli in latent and chronic TB patients and to be the first to apply modern techniques of molecular imaging to the clinical evaluation of novel agents with anaerobic activity against *M.tb*.

NIAID support for TB research has led to significant advances in the understanding of the basic biology, microbiology, and immunology of TB. These advances will foster the development of new diagnostic tools, vaccine candidates, and therapeutic strategies to prevent and ultimately cure this devastating disease.